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Efficiency of ginger extract in controlling biofilm of *Klebsiella pneumoniae*

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ABSTRACT

Administration of increased number and the dosage of antibiotics are resulting in the emergence of microbial strains that are resistant to antibiotics. *Klebsiella pneumoniae* is one such microorganism. The present study was undertaken to investigate the effect of ginger extract on a biofilm of *Klebsiella pneumoniae*. The effectiveness of a standard antibiotic was also checked and the results were compared with those given by ginger extract.

Key words: *Zingiber officinale*, *Klebsiella pneumoniae*, biofilms.

INTRODUCTION

A biofilm is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Biofilms are usually found on solid substrates submerged in or exposed to some aqueous solution, although they can form as floating mats on liquid surfaces. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic. Biofilms are responsible for a number of diseases such as hospital acquired infections. Biofilm contamination and fouling occurs in nearly every industrial water-based process. Major aspects of biofilm research have centered upon their prevention and control in a wide variety of situations. There are number of research workers who have worked upon different aspects of biofilms (Ceri *et. al.*, 1999; Potera, 1999; Stoodley *et. al.*, 2002; Beech and Sunner, 2004 and Harrison *et. al.*, 2005).

Since the last few decades, research work on medicinal plants; either in terms of to purifying active components from the plants, or determining their chemical structure and even trying to chemically synthesize them has increased tremendously due to the emergence of increasing numbers of antibiotic resistant microorganisms.

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines . *K. pneumoniae* are opportunistic pathogens and can cause bacterial pneumonia and some other urinary tract and wound infections, particularly in immunocompromised individuals. *Klebsiella* ranks second to *E. coli* for urinary tract infections in older persons. When infection with *Klebsiella* organisms occurs in the lungs, they cause destructive changes such as necrosis, inflammation, and hemorrhage within the lung tissue. In recent years, *Klebsiella* have become important pathogens in nosocomial infections. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains necessitating the demand of finding new sources of drugs to control them.

Ginger (*Zingiber officinale*) has historically been used in folk medicines of India and China to treat inflammation, control nausea and vomiting and improve digestion. It is reported to have antiemetic effect (Lumb, 1993), it has been used in treating rheumatism (Srivastava and Mustafa, 1992) and to control nausea and vomiting in clinical trials (Earnst and Pittler, 2000). Because of its wide availability and usage, ginger was selected for the present experiment.

MATERIALS AND METHODS

Preparation of extract

5 g ginger was homogenised in mortar and pestle with 5 ml of sterile distilled water. The extract obtained was passed through muslin cloth, squeezed and finally filtered through st. Millipore filter of size 0.45 microns. The filtrate was used for the experiment.

Test organism

Clinical isolate of *Klebsiella pneumoniae* was employed in the present study as the test organism. It was maintained on Nutrient agar slants with periodic sub-culturing and storage in the refrigerator. A suspension was prepared in 0.85% saline, washed twice with saline and was adjusted to 0.1 O.D. at 530 nm.

Experimental

Three sterile beakers containing 5 ml nutrient broth and 0.5 ml 10% glucose were taken and labeled as Control, test G (Ginger test) and test C (Ciprofloxacin test). In Control, 1 ml culture of *Klebsiella pneumoniae* was inoculated. In test G, 0.5 ml of ginger rhizome water extract was added to the beaker in addition to the 1 ml *Klebsiella pneumoniae* culture. In case of test C, along with 1 ml culture, 0.5 ml of Ciprofloxacin was added instead of ginger extract. Alcohol sterilized 12 cover slips were put in each beaker and incubated at 37º C. Two cover slips were removed from each beaker after a period of 2,4,6,8,10 and 24 hours and fixed onto slide with DPX, followed by Gram staining, and Allison and Sutherland staining. While performing Gram staining, as the biofilm cells are already fixed to the surface a care was taken during the heat fixing. This was done by placing a cover slip scraped with petroleum jelly on to the biofilm smear and then carrying out the Gram staining as per the normal protocol.

RESULTS AND DISCUSSION

In case of Control, Gram negative large clumps of cells with lots of matrix and polysaccharides were observed for the first 2-10 hours. By the end of 24 hours, massive matrix with polysaccharides embedded gram negative cells were distinctly seen (Fig.1a). In case of test G, small

Fig. - 1: Control - showing large clumps of cells and polysaccharide

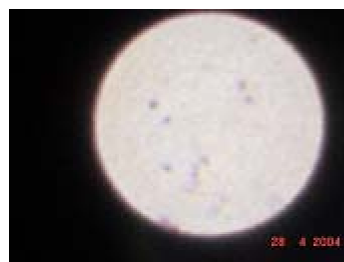

Fig. - 2: Test G (Ginger test) - showing negligible amount of polysaccharide and matrix

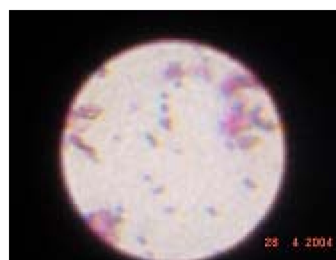

Fig. - 3: Test C (Ciprofloxacin test) - showing clumps of cells and polysaccharide lesser compared to cotrol but far greater than observed in Test G.

clumps of cells with negligible matrix and small amount of polysaccharide could be seen till the end of 10 hours. At the end of 24 hours incubation, no clumps of cells and no matrix were observed along with minuscule amount of polysaccharide (Fig.1b). Test C showed larger clumps of cells with greater matrix and polysaccharide than seen in case of test G, but lesser than the Control by the end of 10 hours. Observations at the end of 24 hours showed negligible cells and matrix. Polysaccharide was still could be observed (Fig.1c).

As the beakers were kept for incubation the *Klebsiella pneumoniae* starts to grow producing capsule and a matrix of polysaccharide and clumped cells which were observed in case of control. But as the ginger extract was added to the beaker it reduces the polysaccharide, reduces the clumps and the matrix suppressing the growth of cells as seen in test G after both gram staining and Allison and Sutherland staining. Allison and Sutherland staining (Allison and Sutherland, 1984) is a special staining technique that utilizes Congo red stain in which bacterial cells stain dark red and exopolysaccharide stains orange-pink. Test C suggests that the antibiotic Ciprofloxacin kills the cells but is not capable of removing the polysaccharide.

CONCLUSION

The present study shows that Biofilms can be artificially constructed by employing a simple and economical set-up in the laboratory. The results clearly indicate the efficacy of ginger extract in controlling the biofilm and the polysaccharide. Although ginger does not seem to inhibit the formation of polysaccharide, its application to the pre-formed biofilm stimulates the deterioration of the biofilm and also suppresses the cell growth. So the purified extracts of ginger can be used both in rural areas where costly drugs are unaffordable and in the urban areas where emergence of increasing numbers of antibiotic resistant microorganisms is becoming a menace. Other advantages of using ginger are it is easily and widely available, cheap and without any side effects.

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